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Chemical composition, safety and quality attributes of traditional cottage sausage

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Abstract: The characteristic features of traditional cottage sausage were analysed. In addition, the extent to which manufacturers create product diversity on the market was investigated, along with potential health risks of the product to consumers. The samples had high overall sensory scores. The average level of fat slightly exceeded 28%, cholesterol content was in the range of 435.4–1220.3 mg/kg and salt content was 1.53–2.77%. Some manufacturers do not cure their product, but about 20% of them apply nitrites above the level of 150 mg/kg. Due to their relatively high pH level (5.76–6.60) and water activity (0.95–0.98), Polish cottage sausage can be a medium which encourages the growth of microorganisms; however, pathogenic bacteria were not found. Histamine was detected in only 42% of the samples, at the low level of 2.6 to 34.2 mg/kg. Principal Component Analysis (PCA) was applied and the dominant variables were specified for particular PCs.

Keywords: biogenic amines; chemical composition; food safety; food quality; microbial contamination; sausage; traditional food

Problems relating to regional and traditional food products are frequently discussed in various circles. However, these are mainly concerned with product identification, marking, registration and promotion, with less emphasis on quality and safety issues. Contrary to popular opinion, there can be numerous quality defects in such products, which may even pose a health risk to consumers (Toldrá 2005). Compared with modern food technology, traditional methods of production are less likely to meet hygiene requirements and maintain appropriate technological parameters (Kołożyn-Krajewska 2008). It is also believed that adjusting methods of production to comply with all legal requirements may deprive such products of their specific character (Jordana 2000). The European Union has taken steps to protect the origin and designations of such foodstuffs in order to guarantee their quality and original character and to give them legal protection.

Sausages are manufactured in virtually all European countries. Their great diversity is due to the different raw materials used and the technologies applied, however, there is still continuous consumer demand for traditional sausages (Marcos *et al.* 2016). The 'Tradisausage' project (2003–2006) promoted the quality and safety aspects of traditional sausages from 6 European countries: France, Greece, Italy, Portugal, Spain, and Slovakia (Talon *et al.* 2007; Latorre-Moratalla *et al.* 2008). Polish cottage sausage (wiejska kiełbasa) is one of the most recognizable traditional products in this country. Since such sausage is produced often in small or medium meat processing plants it is more difficult to achieve the required level of quality control in such areas as

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chemical composition, microbial contamination and the presence of biogenic amines formed, *inter alia*, by the activity of bacterial decarboxylases on amino acids (Latorre-Moratalla *et al.* 2008; Modzelewska-Kapituła & Maj-Sobotka 2014). Moreover, recent studies showed that sausages smoked by traditional technique contained significantly more polycyclic aromatic hydrocarbons (PAHs) as compared to the products smoked by the industrial manner (Zachara *et al.* 2017). It is therefore highly likely that traditional products, such as cottage sausage, may sometimes be of inferior quality, and may even pose a risk to consumers.

Since there is still a lack of information in the literature on this issue, the aim of this paper is to characterize the composition of traditional meat product known as cottage sausage (wiejska kiełbasa) and to indicate the degree of differentiation among the products obtained from various producers. The research also sought to determine to what extent these sausages could pose a health risk to consumers and what actions should be taken to reduce these risks.

MATERIAL AND METHODS

Material. The investigated material consisted of cottage sausage produced in 24 randomly selected small and medium-sized meat processing plants in the southern region of Poland during the autumn-winter season. Each of these plants applies traditional recipe and technology. The sausage is a medium-minced meat product made of pork meat and fat, occasionally with small amounts of beef added, stuffed into natural small intestine casings. Sometimes the sausage is mildly cured. It also contains salt and usually pepper and garlic. After stuffing, the sausage is hung at 20–30°C and then undergoes smoking. The parameters of this process vary slightly among different producers; however, typically the surface is dried at 40–60°C in low density smoke as the first phase, followed by smoking at 45–60°C, with the smoke density ranging from medium to very high. The final phase, which is conducted at 75–85°C in low density smoke, is intended to denature the proteins in the stuffing. At the end of the smoking process, sausages are cooled in cold air for several minutes to lower their internal temperature to about 40°C. After manufacturing, sausages were chilled to 6 ± 1°C and taken randomly three times from each plant, at several day intervals for analysis. Part of each sample, with the skin removed, was comminuted using a meat grinder with a 2.5 mm mesh, while the remaining part was not minced prior to analysis.

Chemical analyses. The basic chemical composition was determined according to International and Polish standards: water and protein by drying at 103°C and Kjeldahl method respectively, fat by the Soxhlet method, and ash at 550°C. Sodium chloride was assayed by Mohr method and nitrate(III) spectrophotometrically at 540 nm after reaction with sulfanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride. The amino nitrogen content (ANC) was analysed by a modified Pope and Stevens method (Fik & Surówka 1984). Cholesterol concentration was determined by an enzymatic method using a commercially available kit (No. 10 139 050 035; Boehringer Mannheim/R-Biopharm, Germany). Measurements of pH were carried out using an HI 9321 pH-meter (Hanna Instruments; Woonsocket RI, USA), while water activity was analysed using a LabMaster-aw meter (Novasina, Switzerland).

Determination of biogenic amines. Biogenic amines analysis was carried out using the HPLC method (Özogul *et al.* 2002). LaChrom HPLC apparatus (Merck, Germany) equipped with an ACE 3 C18, 150 × 4.6 mm (ACE; Cadds Ford, USA) column was employed.

Minced sausage samples were homogenised with 6% trichloroacetic acid (TCA) and filtered. Then derivatisation was conducted with benzoyl chloride, and the whole was extracted with diethyl ether. After its evaporation the residue was dissolved in acetonitrile. The injection volume was 10 µl and detection was monitored at 254 nm. The gradient elution programme, in which HPLC grade acetonitrile (A) and water (B) were used, was as follows: 0–13 (40–75% A and 1.0 ml/min), 13–16 (75–100% A, 1–1.5 ml/min), 16–20 (100% A, 1.5 ml/min), and 20–25 min (100–40% A, 1.5–1 ml/min).

Quantitative analysis of biogenic amines (putrescine, cadaverine, histamine, tryptamine, tyramine, spermine, and spermidine) was carried out using a standard curve for each amine in the range of 0–50 µg/ml (Özogul *et al.* 2002). The total level of biogenic amines (TLBA) was calculated as the sum of the concentrations of the above-mentioned amines.

Microbiological analyses. All microbiological enumerations were determined according to International Commission on Microbiological Specifications for Foods (ICMSF). The total number of aerobic mesophilic bacteria (TVC), was determined

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on plate count agar medium after incubation at 30°C for 72 hours. The level of lactic acid bacteria (LAB) was assayed by applying MRS medium (at 30°C for 72 h). Determination of the *Enterobacteriaceae* count was performed on crystal violet neutral red bile glucose agar (VRBG) incubated at 37°C for 24 h, while detection of yeasts and moulds was conducted on plates with Sabouraud agar medium incubated at 25°C for 5 days. Moreover, *Salmonella sp*. and *Listeria monocytogenes* were detected.

Sensory evaluation. In the sensory evaluation, conducted using a 5-point scale (1 and 5 – the lowest and the highest score respectively), the following attributes of sausages were assessed: surface and cross-section appearance, texture, juiciness, odour and taste. Each evaluation was carried out by nine trained panellists. For particular quality attributes, scores given by the nine panellists were totalled and the means were calculated. The overall sensory quality (OSQ) for sausage was calculated as the mean of scores for all particular parameters.

Instrumental textural analysis. Measurements of sausage texture were performed using a TA-XT2 Texture Analyser (Stable Micro Systems, UK). Cylinders 22 mm in diameter and 3 cm long were subjected to the texture profile analysis (TPA) (Breene 1975). The samples were compressed twice with an SMS P/45 plunger to a depth of 15 mm, at a rate of 2 mm/s and the time between strokes was 10 seconds. The apparatus recorded the force exerted by the plunger as a function of time, from which brittleness, hardness, cohesiveness, springiness and chewiness were determined.

Statistical analysis. Analyses were performed in triplicate, whereas textural measurements were done in nine replications. The results obtained were analysed statistically using the CSS Statistica 12, (StatSoft, Inc., USA) package. Mean values were calculated for each sausage examined, on the basis of which mean values and standard deviations were determined for all the sausages as well as minimum and maximum values. Correlation coefficients ($P < 0.05$) and principal component analysis (PCA) were used to analyse relationships between variables.

RESULTS AND DISCUSSION

Chemical composition. The results of the basic chemical composition of the traditional Polish cottage sausages are given in Table 1. Water was found to be the component occurring in the greatest quantity.

Table 1. Chemical composition of cottage sausage

	Moisture	Protein	Fat	Ash	NaCl	ANC	Nitrites	Cholesterol	pH	Water activity
	(%)						(mg/kg)			
Mean ± s.d.	49.02 ± 5.64	20.48 ± 2.36	28.32 ± 5.99	2.57 ± 0.35	2.07 ± 0.33	0.39 ± 0.07	124.5 ± 96.7	694.7 ± 163.0	6.15 ± 0.21	0.96 ± 0.01
Min	39.73	14.15	16.51	1.95	1.53	0.26	19.6	435.4	5.76	0.95
Max	58.42	24.63	40.14	3.22	2.77	0.50	347.7	1220.3	6.60	0.98

ANC – amino nitrogen content

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The protein content in each case exceeded 14%, and the amino nitrogen constituted approx. 12% of total nitrogen. There was a distinct negative correlation between fat and moisture content ($r = -0.88$, $P < 0.05$), whereas ash correlated well with NaCl concentration ($r = 0.91$, $P < 0.05$).

The study revealed that most manufacturers apply curing at a level necessary to achieve the desired processing effect, but about 20% of them significantly exceed the level of 150 mg/kg. Dżugan and Pasternakiewicz (2007) found the highest nitrate content in salami type sausages and cottage ham, while Michalski (1998) detected excessive levels of nitrates and nitrites in 9% of scalded sausages. The sausages examined varied quite widely in cholesterol content, the *coefficient of variation* was as high as 23%.

Starter cultures are not used in the production of cottage sausage, and meat at an early stage of post-mortem transformation is mostly used. As a result, the sausages obtained were just slightly acidic (pH 6.15), much less so than most fermented sausages with typical pH values of 5.5 (Roserio *et al.* 2010). Water activity in the latter is also usually lower (Gençcelep *et al.* 2008); in the cottage sausages analysed this value ranged from 0.95 to 0.98, as in other Greek and Polish sausages (Ambrosiadis *et al.* 2004; Malicki & Brożewicz 2004). Therefore, taking into account the pH value and water availability, cottage sausage provides a favourable medium for the growth of microorganisms during storage.

Microbial contamination and biogenic amines profile. Since traditional cottage sausage, wiejska kiełbasa, is intended for direct consumption without additional thermal treatment, microbiological safety is particularly important, especially due to its chemical composition, relatively high pH and water activity, which create an environment able to encourage the growth of microorganisms. The total number of aerobic mesophilic bacteria (TVC) was, on average 6.0 log CFU/g, with extreme values of 2.4 log CFU/g and 7.4 log CFU/g. For lactic acid bacteria (LAB), the mean value was 5.2 log CFU/g, with extrema of < 1 and 6.5 log CFU/g, while for yeasts and moulds these values were 3.1 log CFU/g, with extrema of 1 and 4.2 log CFU/g. It should be stressed, however, that no *Enterobacteriaceae* were detected in any of the sausages examined. Moreover, in none of the samples examined did the level of *Listeria monocytogenes* exceed 2.0 log CFU/g and no *Salmonella sp.* were detected in 25 g of product. It is assumed that in medium minced non-fermented sausages, the TVC should not exceed 6.0 log CFU/g, which was the mean value determined in the sausages examined in this work. Similar levels of these bacteria were found in traditional Portuguese Catalão and Salsichão sausages (Laranjo *et al.* 2016).

Lactic acid bacteria (LAB) constitute about 10% of the total count of microorganisms in individual sausages. Their number correlates well with TVC ($r = 0.99$, $P < 0.05$). In fermented sausages, these bacteria are the dominant microflora. For example, in Turkish and Greek sausages maximum levels may be as high as 8.8 log CFU/g (Ambrosiadis *et al.* 2004, Gençcelep *et al.* 2008), while in similar Czech sausages the average level is approx. 7 log CFU/g (Komprda *et al.* 2004). There was also a correlation ($r = 0.64$, $P < 0.05$) between the number of LAB and yeast and mould contamination; the presence of the latter in examined cottage sausages may pose a risk since, given a water activity value of 0.96, these microorganisms may develop in favourable conditions.

Biogenic amines concentrations are presented in Table 2. Histamine was detected at a level of 2.6 to 34.2 mg/kg in sausages originated from 10 of the 24 manufacturers. Such concentrations do not pose a significant threat to human health. Assuming the consumption of sausages at typical portion sizes, there is no danger that histamine intake will exceed 50 mg per meal for one person. This quantity is recognized by the European Food Safety Authority (EFSA) as having no harmful effect on healthy people. However, for people with histamine intolerance, even small amounts can cause serious health problems; only consumption at quantities below the level detectable by recognized methods can be considered safe (EFSA 2011). Research conducted as part of the

Table 2. Biogenic amines concentration in cottage sausage (mg/kg)

	Histamine	Putrescine	Cadaverine	Tryptamine	Tyramine	Spermine	Spermidine	TLBA
Mean ± s.d.	8.7 ± 10.6	17.7 ± 11.9	31.0 ± 35.5	13.3 ± 7.1	10.8 ± 9.3	76.3 ± 29.1	26.1 ± 7.8	185.0 ± 58.6
Min	0.0	6.2	0.0	4.2	0.0	15.9	12.2	91.8
Max	34.2	51.8	107.6	35.0	40.3	127.9	38.6	343.9

TLBA – total level of biogenic amines

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Tradisausage project on 54 different types of matured sausage also showed that only in eleven sausages was histamine content greater than 10 mg/kg (Latorre-Moratalla *et al.* 2008). In two cases, however, its level exceeded 100 mg/kg, which agrees with the findings of Gençcelep *et al.* (2008) who analyzed thirty samples of sucuk sausages.

Putrescine concentration was low; cadaverine, on the other hand, was not detected in two samples, but exceeded 100 mg/kg in two other samples. In comparison with fermented sausages, which can have levels of cadaverine of up to 611 mg/kg, that was a small and safe amount (Latorre-Moratalla *et al.* 2008). The content of tryptamine and tyramine can also be considered safe. The former was detected in all samples. However, its amounts were no greater than in fermented sausages (Gençcelep *et al.* 2008; Latorre-Moratalla *et al.* 2008). The latter was present in only half of the sausages examined, in which its content did not exceed 40.3 mg/kg; this was distinctly lower than in fermented sausages from France, Spain, Italy, Greece, Portugal, Slovakia (Latorre-Moratalla *et al.* 2008) and Turkey (Gençcelep *et al.* 2008) where the highest levels were close to 700 mg/kg. According to the EFSA (2011), a tyramine content of 600 mg/kg is safe for healthy consumers. However, for individuals taking third generation monoamine oxidase inhibitor (MAOI) drugs, amounts as low as 50 mg/kg can be harmful.

Spermine and spermidine were present in all the samples. To date, however, no evidence has been found that these amines have a detrimental effect on human health. The lowest level of total biogenic amines (TLBA) was about 90 mg/kg, whereas the highest was almost 4 times as much, indicating considerable diversity in the sausages manufactured by different producers. The initial quality of the raw materials, levels of hygiene and conditions of distribution play a major role for the content of biogenic amines in sausages, affecting microbial contamination and the rate of biochemical processes.

Sensory evaluation and instrumental texture analysis. Most of the sausages examined obtained high sensory scores for individual quality indicators and OSQ (Figure 1). The less favourable taste evaluation of some sausages resulted from excessive saltiness, whereas odour in few samples was assessed lower due to insufficient intensity resulting from inappropriate smoking.

The results of texture measurements according to the instrumental TPA procedure are given in summary form in Table 3. The crumbly texture and relatively low mean values for hardness and cohesiveness and moderate for springiness may be related to the higher pH than matured sausages. As was reported by Spaziani *et al.* (2009), low acid sausages are characterized by low hardness and cohesiveness. All the texture parameters analysed instrumentally are correlated to each other. The highest correlation was found for chewiness ($r \geq 0.92$; $P < 0.05$). Therefore it can be regarded as most representative of the texture of the sausages examined.

Principal component analysis. The loading plot (Figure 2A) demonstrate that parameters referring to the composition, especially moisture, fat, ash, amino nitrogen and NaCl content, as well as overall sensory quality were important variables for the first principal component (PC1). The dominant variables for the second component (PC2) are water activity and nitrites, although some other variables are also of significance. The score plot (Figure 2B) on

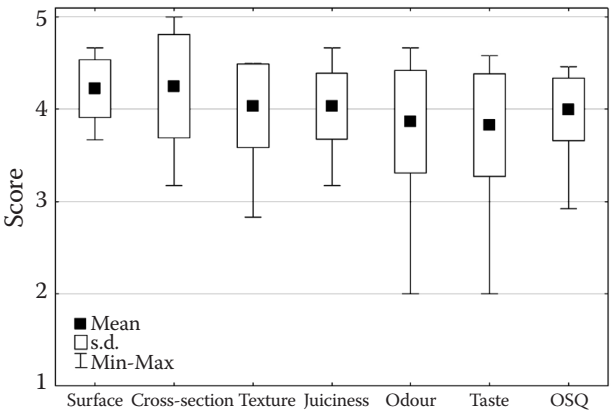

Figure 1. Sensory characteristics of traditional cottage sausage

Table 3. Instrumental texture characteristics (TPA) of cottage sausage

	Brittleness	Hardness	Chewiness	Cohesiveness	Springiness
		(N)			
Mean ± s.d.	23.25 ± 9.35	25.26 ± 13.39	5.30 ± 6.87	0.280 ± 0.092	0.553 ± 0.12
Min	14.24	11.43	1.11	0.167	0.351
Max	48.79	66.80	31.81	0.596	0.798

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the axes of the first two PCs indicates the uneven distribution of those sausages which have different properties. More detailed PCA findings are presented in Tables S1 and S2, see Electronic Suplementary Material (ESM).

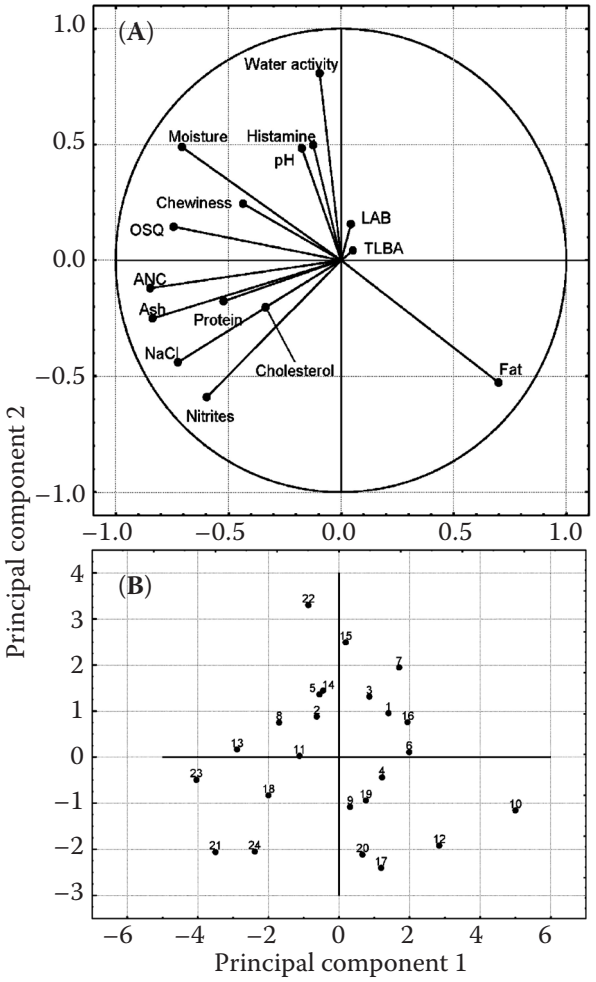

Figure 2. PCA loading (**A**) and score (**B**) plots in the plane of PC 1 vs. PC 2. The labels correspond to variables examined and to particular cases (sausages) respectively

CONCLUSIONS

Producers of traditional Polish cottage sausage, wiejska kiełbasa, provide products varying in their chemical composition, particularly fat, salt, cholesterol, and nitrite content. Although the average content of these ingredients is generally acceptable, it is advisable that some manufacturers should modify their recipes to reduce them. These products are characterized by high average sensory quality, with a unique flavour and aroma and typically crumbly texture. Due to relatively high water activity and pH compared with fermented sausages, Polish cottage sausage could potentially provide a favourable environment for the growth of pathogenic microorganisms. However, in practice these do not occur, despite a fairly high total number of mesophilic bacteria. Nevertheless, such products should be cold stored. Although histamine was found in some sausages, its level did not pose a threat to human health. The content of the remaining biogenic amines was low and can be considered safe. The results obtained, supported by the principal component analysis (PCA), indicate chemical, microbiological and textural variation among the sausages. Nevertheless, they are a safe and attractive meat product that meets quality requirements.

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